

Application No. 09/546,857
Amdt. dated November 22, 2004
Reply to Office Action of July 22, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claims 1-57 (canceled)

Claim 57 (new): A variant of human VEGF comprising amino acid substitutions D63S, G65M, and L66R.

Claim 58 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 57.

Claim 59 (new): A vector comprising the nucleic acid of claim 58.

Claim 60 (new): A variant of human VEGF comprising one or more non-conservative amino acid substitution(s) at residues 63 to 66 and one or more amino acid substitution(s) at residues 18, 21, 22, or 25, wherein the VEGF variant exhibits selective binding affinity for KDR receptor.

Claim 61 (new): The VEGF variant of claim 60, wherein the amino acid substitution(s) comprises D63S, G65M, or L66R.

Claim 62 (new): The VEGF variant of claim 60, wherein the amino acid substitution(s) comprises M18E, Y21L, Q22R, or Y25S.

Claim 63 (new): The VEGF variant of claim 60, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 64 (new): The VEGF variant of claim 60 wherein the amino acid substitutions comprise D63S, G65M, and L66R.

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Claim 65 (new): The VEGF variant of claim 64, wherein the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 66 (new): The VEGF variant of claim 64, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 67 (new): A VEGF variant of claim 60, comprising one of the following combinations of amino acid substitutions:

- (a) M18E, D63S, G65M, and L66R;
- (b) Y21L, D63S, G65M, and L66R;
- (c) Q22R, D63S, G65M, and L66R;
- (d) Y25S, D63S, G65M, and L66R;
- (e) M18E, Y21L, D63S, G65M, and L66R;
- (f) M18E, Q22R, D63S, G65M, and L66R;
- (g) M18E, Y25S, D63S, G65M, and L66R;
- (h) Y21L, Q22R, D63S, G65M, and L66R;
- (i) Y21L, Y25S, D63S, G65M, and L66R;
- (j) Q22R, Y25S, D63S, G65M, and L66R;
- (k) M18E, Y21L, Q22R, D63S, G65M, and L66R;
- (l) M18E, Q22R, Y25S, D63S, G65M, and L66R;
- (m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;
- (n) M18E, Y21L, Q22R, Y25S, and D63S;
- (o) M18E, Y21L, Q22R, Y25S, and G65M;
- (p) M18E, Y21L, Q22R, Y25S, and L66R;
- (q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;
- (r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;
- (s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or
- (t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

Claim 68 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 60.

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Claim 69 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 63.

Claim 70 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 65.

Claim 71 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 67.

Claim 72 (new): A vector comprising the nucleic acid of claim 68.

Claim 73 (new): A host cell comprising the vector of claim 72.

Claim 74 (new): A composition comprising the VEGF variant of claim 60 and a carrier.

Claim 75 (new): The composition of claim 74, wherein the carrier is a pharmaceutically acceptable carrier.

Claim 76 (new): An assay for detecting KDR receptor, comprising contacting an isolated cell or tissue with a VEGF variant of claim 60 and assaying for binding of the VEGF variant to the cell or tissue.

Claim 77 (new): A method for stimulating phosphorylation of a KDR receptor, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of the KDR receptor.

Claim 78 (new): A method for stimulating MAP kinase activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of MAP kinase.

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Claim 79 (new): A method for stimulating PLC-gamma activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of PLC-gamma.

Claim 80 (new) A method for stimulating PI 3'-kinase activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of PI 3'-kinase.

Claim 81 (new): A method for stimulating vasculogenesis or angiogenesis, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 60.

Claim 82 (new): A method for promoting the migration of endothelial cells, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 60.

Claim 83 (new): A variant of human VEGF comprising two or more amino acid substitutions at residues 17 to 25, wherein the VEGF variant exhibits selective binding affinity for KDR receptor.

Claim 84 (new): The VEGF variant of claim 83, wherein the amino acid substitutions comprise two or more amino acid substitutions at residues 18, 21, 22, or 25.

Claim 85 (new): The VEGF variant of claim 83, wherein the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 86 (new): The VEGF variant of claim 83, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

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Claim 87 (new). The VEGF variant of claim 83, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22K, and Y25S.

Claim 88 (new): The VEGF variant of claim 83, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22E, and Y25I.

Claim 89 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 83.

Claim 90 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 86.

Claim 91 (new): The VEGF variant of claim 60, wherein the amino acid substitutions further comprise a substitution at residue 17.

Claim 92 (new): A variant of human VEGF, comprising:

- (a) one or more amino acid substitution(s) at residues 17-25, and
- (b) one or more amino acid substitution(s) at residues 63-66;

wherein amino acid residue 60 is cysteine.